Nested Frequency Plot Sample Design and Field Methods for Vegetation Monitoring

Materials
Plot frame
Map of site showing boundaries and sampling point locations
Compass
GPS pre-loaded with sample points
Clipboard
Pencils
Data sheets
Identification guides and/or keys
Plant press
Hand lens
List of species planted and/or list of species previously found (optional)
Camera to photograph unknown plants

Introduction
 Frequency has been long recognized as a reliable and repeatable measure of plant abundance for purposes of monitoring (Curtis and McIntosh 1950, DeBacker et al. 2011). It avoids observer bias associated with cover estimates. By nesting plots of different sizes, it can be used to assess trends in species that vary substantially in relative abundance. Frequency is sensitive to the overall distribution of each species, but simulation (Heywood and DeBacker 2007) and field (DeBacker et al. 2011) studies have demonstrated that a plot size that results in frequencies of 20 – 50% (35% is a good target) provides the greatest statistical power for assessing change over time. This frequency refers to the proportion of plots in which a species is detected within a reconstruction. For example, big bluestem may be dominant at a reconstruction, resulting in detection in every 0.5 x 2 m plot, making it impossible to assess any increase. However, it may be detected less often in a smaller plot size, perhaps 0.25 x 0.125 m. To assess changes in frequency over time of big bluestem in this example, the smaller plot frame size should be used to calculate frequency. When summarizing the data, use the plot frame size that yields approximately 35%.
 In addition to monitoring, frequency has also been used to compare plant species occurring in different treatments, such as disturbance types, and within vegetation types (Larson et al. 2001, Larson 2003). Indices, such as species diversity and evenness, also can be calculated from frequency data. The drawback to any protocol that uses plots on transects is that inevitably, some species will be missed. For the most complete list of species occurring at a site, use of both the Nested Plot and the Meandering Walk Protocol is preferable.

Training
Field personnel. Sight-identification of species likely to be encountered (e.g., species planted, regional native and nonnative annuals and "weedy" species), as well as all regional invasive species, is necessary as a starting point. Those using this technique should also have familiarity with plant keys and methods to collect and press specimens for vouchers and identification. The ability to navigate to specific locations using a GPS is required.

Mapping

Prior to the field season, collect the GIS layers for the boundary of the site. If the planting or management did not take soil type into account, it may also be helpful to use a soil survey layer to separate wet, mesic, and dry prairie types. Soils data is available from the Natural Resources Conservation Service Web Soil Survey (NRCS WSS; https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm). We used soil drainage class as a proxy for wet, mesic, and dry prairie types; see Appendix 1 for details on obtaining this data.

Create a map that includes the site boundaries. Create the systematic grid for the appropriate number of sample points (see Appendix 2 for ArcMap tool) and download the points to a GPS unit. It is helpful to include soil polygons or topography on a satellite imagery base map for use in the field. The map and/or shapefile should be stored with the copy of the datasheets and data.

The nested plot protocol, like the meandering walk protocol, is adapted from the Prairie Reconstruction Initiative (PRI) protocol, which was developed to assess changes in vegetation species frequency over time in prairie reconstructions, but works equally well in remnant prairie systems. There are two options when randomly selecting locations to sample, prior to the actual survey. The first involves generating random points within the management area using XTools Pro within ArcMap, and placing transects through these points, parallel to any elevation gradient. In Minnesota, obtain topography information from https://www.dnr.state.mn.us/maps/mntopo/index.html. If there is no elevation gradient, use a random compass bearing to determine the direction of the transect. The total length of transects is based on the area of the prairie type within the management area:

Transect length = (x * 7.8m) + 20m
Where x = 99.8263*(1-exp(-0.163 * management unit area in hectares))

The total number of vegetation plots (0.5 x 2.0 m) to sample is also based on the area of the prairie type within the management area, not to exceed 30 plots:

#plots = 30 * (1-exp(-0.2 * management unit area in hectares))

The PRI protocol chooses sample locations on a systematic grid. The number of plots in each seed mix or management area depends on the size of an individual reconstruction or remnant, similar to the random points method. Between 10 and 40 rectangular plots (0.5 x 2.0 m) are placed in a systematic grid throughout the site. Aim for 3 plots/ha with a minimum of 10 plots and a maximum of 40 plots. In other words, if a site is 3 ha (7.4 acres) or less, use 10 plots. For sites from 4–13 ha, use 3 plots/ha, and if a site is more than 13 ha (32 acres), use 40 plots. The number of plots is based on simulations using test data. The Appendix contains an annotated script in R for creating the systematic grid within ArcMap.

If GIS technology and a GPS unit are not available, the systematic grid will need to be laid out by hand on an appropriate satellite image of the site. An alternative method of navigating to each point will need to be developed (e.g., using compass direction and distance from an obvious starting point) and documented.

Plot frame and data sheet

The plot frame (Figure 1) is made from 0.5 inch PVC pipe, which can be obtained at most hardware stores. The size of the subunits within the plot frame doubles with each larger size. They can be modified, depending on the vegetation characteristics of the site to be monitored, as long as sizes are in multiples of 2 (e.g., the smallest subunit in Figure 1 is 50 cm x 12.5 cm, so the next smaller that could be added is 25 cm x 12.5 cm). The rectangular shape also needs to be retained because species captured varies with plot shape. Keeping in mind the 35% target, a site that is heavily dominated by a single species will need a smaller subunit size than a site that has greater alpha diversity. In tall vegetation, it is useful to be able to remove one side of the frame so that it can be slipped into the base of the vegetation.

Two sample data sheets are attached to this protocol (Appendix 3). The key thing to note is that each species is entered *only once* at each sample plot location (numbers 1-10 across the top of the spreadsheet) and the smallest subunit (1-5) in which it is found is entered under the appropriate plot number.
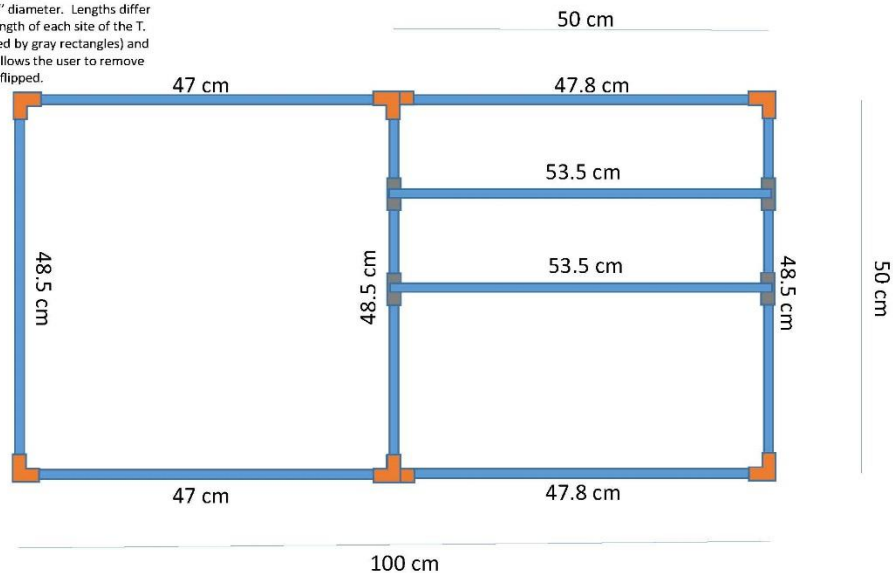

Figure 1. Nested plot frame. Subunit dimensions have been used in tallgrass prairie reconstructions and remnants, but in some cases the smallest subunit may need to be halved if any species are found to occur in > 50% of the 12.5 x 50 cm subunit.

Field methods

Methods
Using a hand-held GPS, navigate to the first plot location to be sampled. Select a compass direction at random and orient the plot in the same way with respect to that direction at each sample location. Alternatively, select a compass direction before going to the field and use it for all sampling. The goal is to avoid biasing plot placement.

Carefully adjust the vegetation so that only plants rooted within each subunit division are recorded. Record all native and nonnative species in the smallest subunit and place a 1 in the corresponding box of the data sheet. Continue inspecting subunits in order from smallest to largest, and record new species along with the subunit in which they first occurred on the data sheet. Each species that occurs in a given 1.0 m^2 plot will only be recorded once by writing the smallest subplot in which it occurred on the data sheet. The final subunit is always the 50 cm x 100 cm area of the plot frame after it is flipped on its short axis from left to right.

If you cannot identify a plant to species, give the highest level of detail possible or collect a sample for later identification. Ignore very small seedlings that cannot be identified with confidence.

Analysis

If changes in frequency of individual species over time is an objective, initial analyses should focus on determining optimal subunit size (35% frequency is a good target (DeBacker et al. 2011)) for each species of interest. The nested feature of the plots should be retained throughout monitoring so that optimal subunit size can be reassessed each sample period. Optimal subunit size indicates the abundance of species, with a smaller optimal subunit size indicating greater abundance (Curtis and McIntosh 1950). If the initial frequency of a species of interest is between 20 and 50%, it should be possible to track its change in frequency over time, whether it increases or declines.
To determine species richness or other quantitative metrics for the site, use a single subunit size – typically the entire plot so no species are omitted – to calculate frequencies by species.

References
Curtis, J. T., and R. P. McIntosh. 1950. The interrelations of certain analytic and synthetic phytosociological characters. Ecology **31**:434-455.
DeBacker, M. D., J. S. Heywood, and L. W. Morrison. 2011. Optimized Frequency Measures for Monitoring Trends in Tallgrass Prairie. Rangeland Ecology & Management **64**:301-308.
Heywood, J. S., and M. D. DeBacker. 2007. Optimal sampling designs for monitoring plant frequency. Rangeland Ecology & Management **60**:426-434.

Larson, D. L. 2003. Native weeds and exotic plants: relationships to disturbance in mixed-grass prairie. Plant Ecology **169**:317-333.

Larson, D. L., P. J. Anderson, and W. Newton. 2001. Alien plant invasion in mixed-grass prairie: effects of vegetation type and anthropogenic disturbance. Ecological Applications **11**:128-141.

Appendix 1 -- Using the NRCS Web Soil Survey to Identify Soil Drainage Classes within a Survey Area

This SOP describes the basic steps you would use to find the soil drainage classes for a particular survey area using the Natural Resources Conservation Service's website.

1. Go to NRCS Soil Survey page: https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm
2. Click the green circular "Start WSS" button or click "Start Web Soil Survey" blue text
3. Click the "Area of Interest (AOI)" tab to define your map location
   a. Zoom in on the map of the US or use the State/County dropdowns on the left and click "View" to zoom in; continue to zoom until your survey area more or less fills the view
   b. Use the polygon AOI tool or the rectangle AOI tool to outline your area of interest (i.e., seed mix area that you plan to monitor)
   c. To define a new AOI for a subsequent map, click "Clear AOI", then redo the above steps
4. Click the "Soil Map" tab to see the outlines of the soil polygons within your AOI. You can change the layout on your screen by choosing one of the two buttons just above the map and to the right -- either normal map layout or full width map layout. With normal layout, the table with the soils descriptions is to the left of the map. With full width layout, it is below the map.
   a. You can click on a soil name to see a description of that soil (map unit).
5. Click the "Soil Data Explorer" tab to visualize properties of the soils within your AOI, specifically drainage class. Expand "Properties and Qualities Ratings" on the left sidebar.
   a. Further expand "Soil Qualities and Features"
   b. Click "Drainage Class"
      i. The Map and Table checkboxes should both be automatically checked
      ii. Click the "View Rating" button to see your AOI with the soil polygons color-coded by drainage class
      iii. Click the "Legend" tab at the upper left of the map to see which color represents which drainage class, and match to dry, mesic, or wet prairie types (Figure 2)
      iv. Figure 3 depicts an example map
6. Click "Download Soils Data" tab to download a shapefile of the soils map of your area of interest, if desired
7. Click "Shopping Cart (Free)" tab to download a report with a printed soils map of your area of interest, if desired
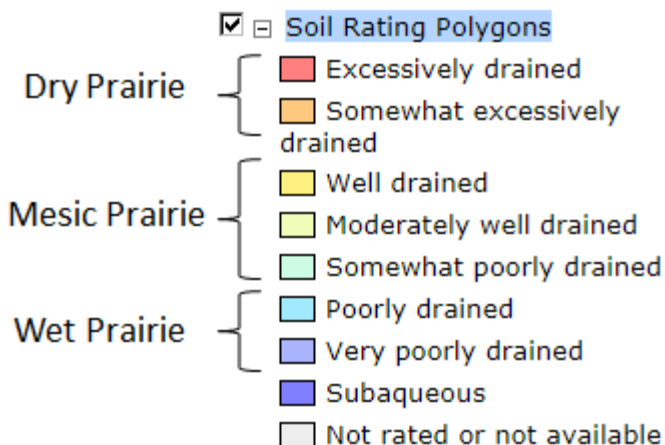
Figure 2. Correspondence of soil drainage class from the NRCS Web Soil Survey ("Soil Rating Polygons" in legend with colored boxes) to prairie types used in this protocol.
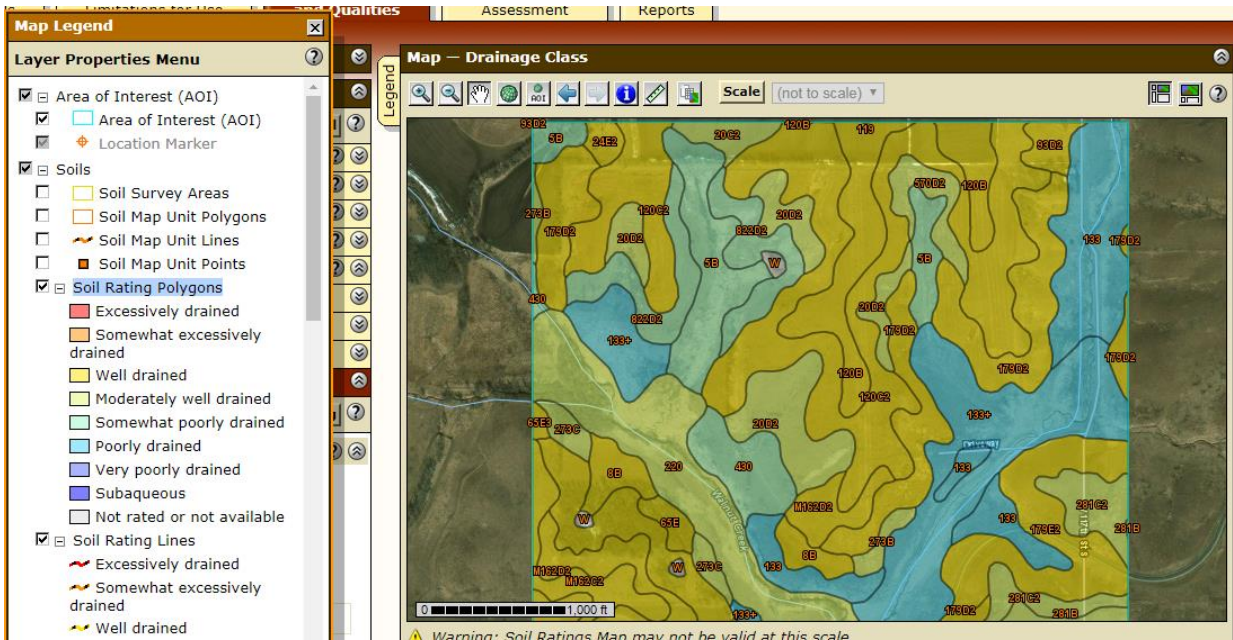
Figure 3. An example of a soils map that can be generated from the Web Soil Survey. Blue = wet, greens and yellow = mesic.

Appendix 2 – Creating the Systematic Grid for Nested Frequency Plots

How to utilize the Monitoring Grid toolbox in ArcMap once you have downloaded and extracted the PRI_Monitoring_Grid.zip file from the PRI site.

1. Open ArcToolbox
2. Right click in ArcToolbox and select "Add Toolbox"
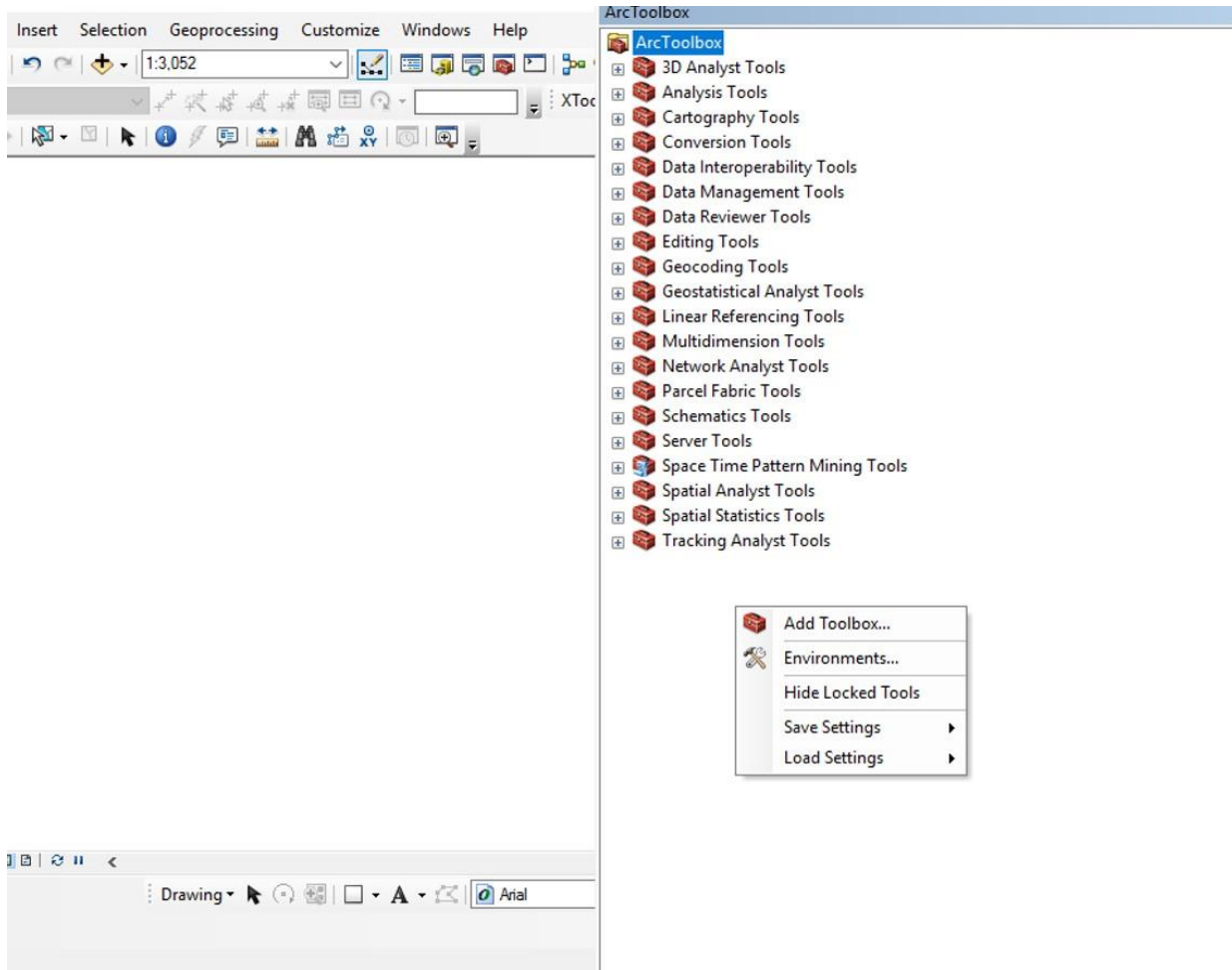
3. Navigate to where you saved the extracted files and select and open the Monitoring_Grid.pyt file.
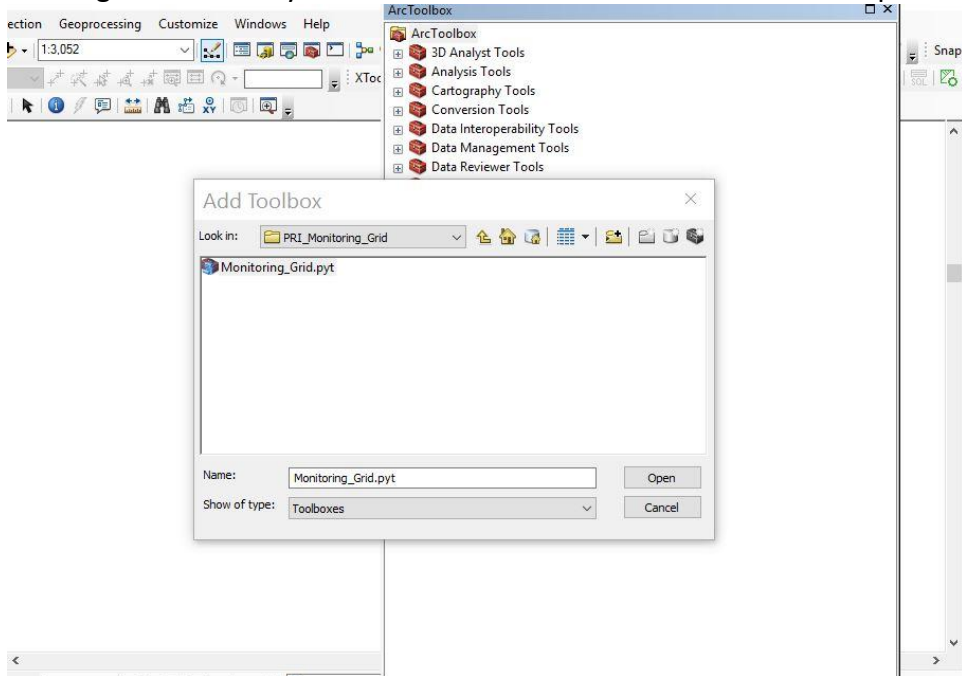
4. Once the Monitoring_Grid folder is added to your toolbox, expand and select the script contained within.
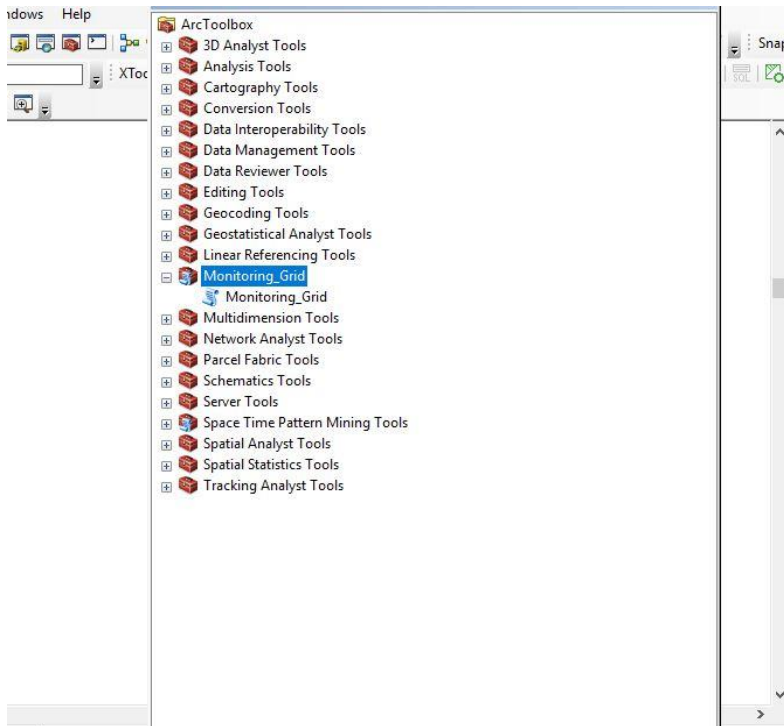
5. The **Input Feature** is the shapefile of the polygon of the area that you want to monitor. This tool is most efficient if the survey area polygon is the only polygon contained within the shapefile. Please contact Ben Walker (benjamin_walker@fws.gov) if you have many survey areas that you want to process in a single event. The **Output File** is the systematic grid that the tool will create, navigate and provide a name to the file.

Workspace is the folder location where the script will temporarily work to create the necessary files and subsequently delete them. On large survey areas, the tool may create more than 40 survey locations; please be aware and delete unnecessary points.
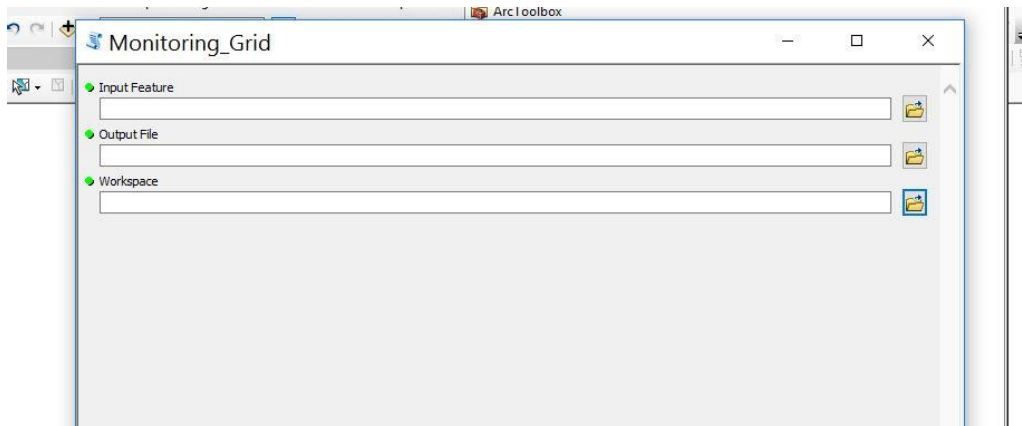
The process that the script runs to create the monitoring grid is outlined below:

```
#Copy the shapefile so we aren't editing it#
arcpy.CopyFeatures_management(inputfile1, "point_cr.shp")
#Add a Square Meter Field and calculate the value#
arcpy.AddField_management("point_cr.shp","area_sqm","Double")          expression1 =
"{0}".format("!SHAPE.area@SQUAREMETERS!")          arcpy.CalculateField_management("point_cr.shp",
"area_sqm", "!SHAPE.area@SQUAREMETERS!", "PYTHON", )
#Define layers for next step#          outraster = "ratemp.tif"          field = "area_sqm"
#Pull area value from polygon#          value = arcpy.da.SearchCursor("point_cr.shp",
("area_sqm",)).next()[0]
#Set the sideboards for the number of sampling points#
size1 = sqrt(value/40)          size2 = sqrt(value/(math.ceil(value/10000)*3))          size3 = sqrt(value/10)


#Convert our layer to a raster based on the sideboards#          if int(value) >= 130000:
arcpy.PolygonToRaster_conversion("point_cr.shp", field, outraster, "", "", size1)
          elif int(value) in range(30000, 130000):
arcpy.PolygonToRaster_conversion("point_cr.shp", field, outraster, "", "", size2)
          else: arcpy.PolygonToRaster_conversion("point_cr.shp", field, outraster, "", "", size3)
          #Convert back to a point shapefile based on each cell's centroid#
arcpy.RasterToPoint_conversion(outraster, outfile2, "Value")
          #Cleanup intermediate files#          arcpy.Delete_management("point_cr.shp","")
arcpy.Delete_management("ratemp.tif","")
          arcpy.AddMessage ("POINTS SUCCESSFULLY CREATED")
```

Appendix 3 -- Data sheet for nested plots protocol

Record the sizes of the subunits used. Don't forget to flip the plot for the final subunit.
Record the smallest subunit within which each species occurred in each plot.

Date:_____ Site ID:_____ Page _____ of _____

Observer:_____ Subunit sizes: (1) _____ (2)_____ (3)_____ (4)_____

(5)_____ (6) _____ (7)_____

Species	1	2	3	4	5	6	7	8	9	10	Comment

Comments: _____

_____

A somewhat different datasheet is on the next page:

Datasheet for Nested Plots Protocol

Record the sizes of the subplots used; use only as many as in the plot. Don't forget to flip the plot frame for the final subplot. Record the smallest subplot within which each species occurred in each plot.

Date:_____ Observer:_____ Overall Site:_____ Planting Site: _____

Seed Mix Area Name: _____ Prairie Type: _____ Page ___ of ___

Subplot sizes (cm) - *Write in dimensions if you did not use these*: (1) <u>12.5 x 50</u> (2) <u>25 x 50</u> (3) <u>50 x 50</u> (4) <u>50 x 100</u> (5) <u>50 x 200</u>

Species	*Plot Number (minimum 10, maximum 40)*										Comment